# Acetylcholinesterase Kinetics

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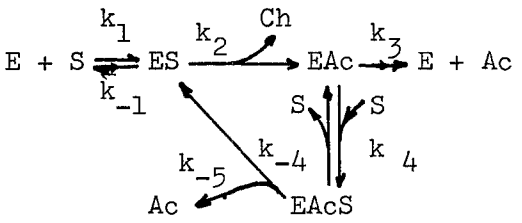
The rate of enzymatic acetylcholine hydrolysis is known to be inhibited by the substrate itself as the concentration exceeds $10^{-3}$ M. Three typical kinetic mechanisms (1,2) are considered in this paper by steady-state analysis, and by numerical integration:
(i) Substrate inhibition occurs through the reaction of acetylcholine with acetylated enzyme. The deacetylation of this ternary complex is supposed to be completely inhibited.
(ii) A ternary complex is formed as in (i). However, the deacetylation is not completely inhibited.
(iii) A two-site-mechanism is discussed where acetylcholine binds either to the active site or to a modifier site. Binding to the latter changes the activity of the active site.
Least squares fits to biochemical constants, such as Michaelis constant $K_m$, inhibition constant $K_i$, maximum rate V, and maximum rate of the ternary complex $V_i$, revealed that mechanism (ii),i.e.




E = Acetylcholinesterase
S = Acetylcholine
Ac= Acetate
Ch= Choline

is the most simple one which can describe satisfactorily the experimental data. The corresponding fitted values are: $K_m = k_3 \cdot (k_{-1}+k_2)/(k_1(k_2+k_3)) = (6.3\pm1.1)\cdot10^{-5}$ M, $K_i = (k_2+k_3)(k_{-4}+k_5)/(k_4 \cdot (k_2+k_5)) = (1.3\pm0.3)\cdot10^{-2}$ M, $V = k_2k_3[E]_t/(k_2+k_3) = k_{cat}[E]_t = 9.7\pm0.4$ I.U., $V_i = k_2k_5[E]_t/(k_2+k_5) = k_{cat_i}[E]_t = 1.0\pm0.4$ I.U., (pH 7.4, 30°C, $[E]_t = (4.7\pm0.6)\cdot10^{-10}$ M). The turnover numbers are found to be $k_{cat} = (1.7\pm0.3\cdot10^4 s^{-1}$, and $k_{cat_i} = (1.8\pm0.9)\cdot10^3$ $s^{-1}$. Limits of the kinetic constants are derived from fitted biochemical constants. The amount of acetylated enzyme in the steady-state was calculated to be 60 % which was confirmed recently by direct measurement (3). Numeric integration of differential equations showed that steady-state approximation can be used in all three cases.

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2. Rosenberry, T.L. and Bernhard, S.A. (1972) Biochemistry 11, 4308 - 4321.
3. Wilson, I.B. (1980) Neurochemistry International, Sept.-Okt., in press.